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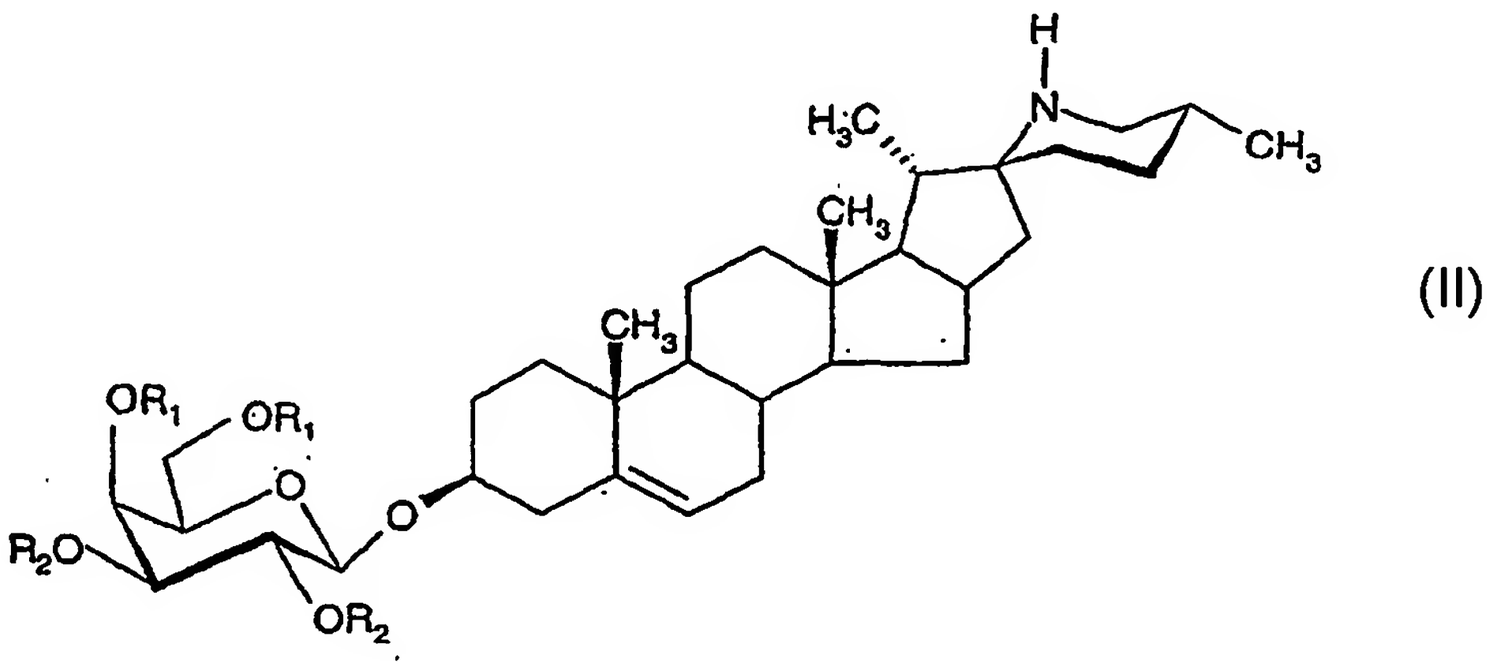
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYNTHESIS OF SOLANUM GLYCOSIDES



(II)

(57) Abstract: The present invention relates to the chemical synthesis of solanum glycosides, in particular to the synthesis of solasonine as well as to novel β-monosaccharide intermediate compounds, of Formula (II), where each R_1 is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group and each R_2 is the same or different and independently represents a benzoyl, acetyl or pivaloyl group.

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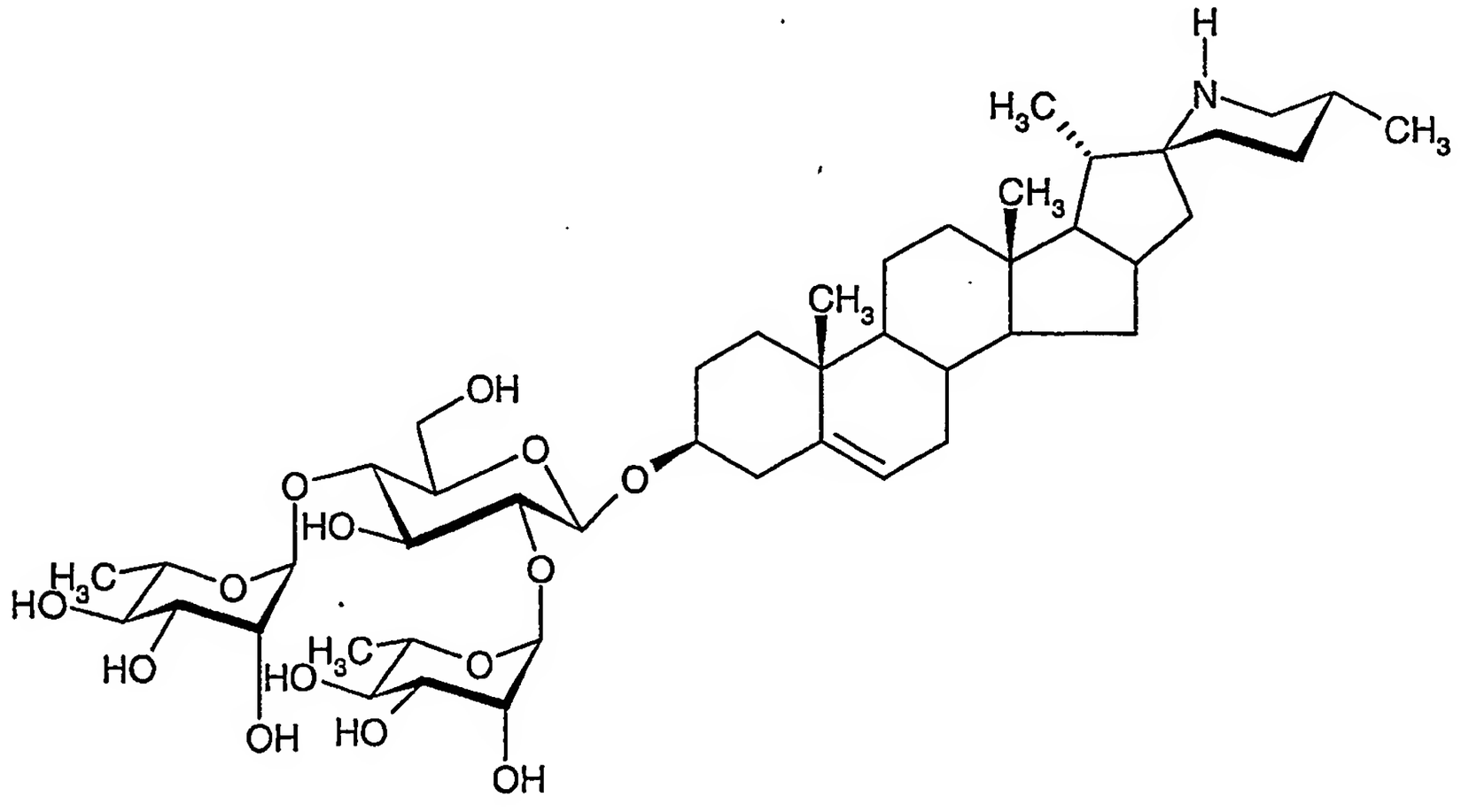
Synthesis of Solanum Glycosides

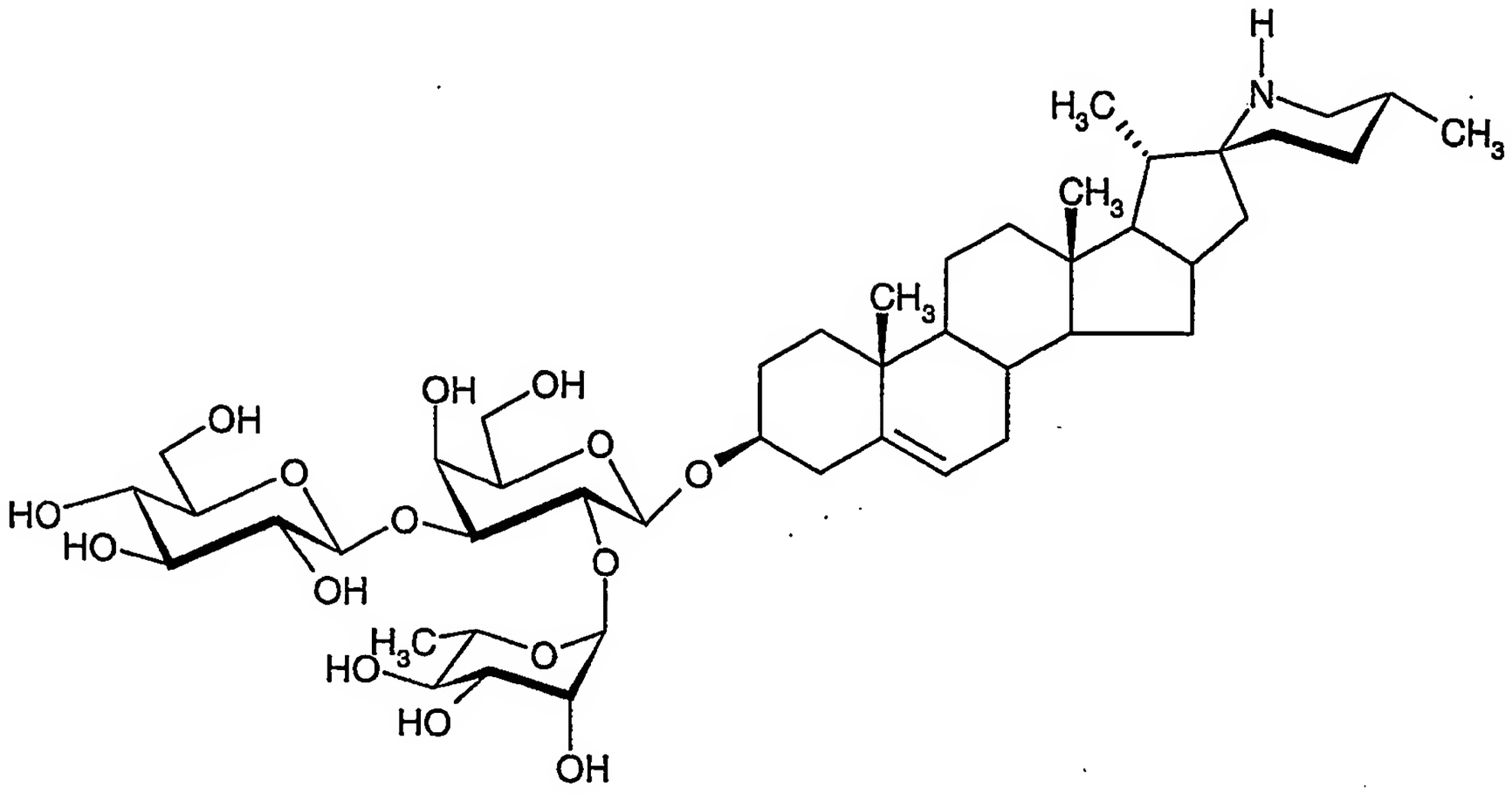
The present invention relates to the chemical synthesis of solanum glycosides, in particular to the synthesis of solasonine as well as to novel β-monosaccharide intermediate compounds.

Solasodine and its glycosides are of considerable interest clinically. They are widely used as starting products for the synthesis of various steroidal drugs, the aglycon solasodine is a source for synthetic cortisone and progesterone.

It is moreover well established that certain naturally occurring conjugate solasodine glycosides have potent antineoplastic properties. Of particular interest are the triglycosides solasonine (22R, 25R)-spiro-5-en-3β-yl-α-L-rhamno-pyranosyl-(1->2 gal)-O-p-D-glucopyranosyl-(1->3 gal)-β-D-galactopyranose and solamargine (22R, 25R)-spiro-5-en-3β-yl-α-L-rhamnopyranosyl-(1->2 glu)-α-L-rhamnopyranosyl- (1->4 glu)-β-D-gluco-pyranose. The structures of these triglycosides are shown below:



Solamargine



Solasonine

The above triglycosides are conventionally obtained by extraction from a plant source. A commercially available extract of *S. sodomaeum*, commonly referred to as BEC (Drug Future, 1988, vol. 13.8, pages 714-716) is a crude mixture of solamargine, solasonine and their isomeric diglycosides. The extraction process for making BEC involves homogenizing the fruits of *S. sodomaeum* in a large volume of acetic acid, filtering off the liquid through muslin followed by precipitation of the glycosides with ammonia (Drugs of today (1990), Vol. 26 No. 1, p. 55-58, cancer letters (1991), Vol. 59, p. 183-192). The yield of the solasodine glycoside mixture is very low (approx. 1%). Moreover the individual process steps are not defined to GMP in terms of scale up, definition of yield, composition and product quality.

There is a great need for a cost efficient process that provides the antineoplastically active triglycoside solasonine at high yield with little or no impurities.

Contrary to other steroid ring systems, the steroid skeleton of solasodine contains a very labile nitrogen-containing ring. This aglycon cannot readily be chemically modified while keeping the steroid skeleton intact. In spite of the fact that the

aglycon solasodine is readily available, the prior art does not disclose the synthesis of the solasonine using the aglycon material as starting material.

The synthesis of solasonine requires the stereoselective glycosylation of solasodine at the relatively unreactive hydroxyl group.

It has been found that solasodine is not compatible with the conventional steroid glycosylation technique. No glycosylation was observed following the treatment of solasodine with tetrabenzoyl α-D-glucopyranosyl trichloroacetimidate and trimethyl-silyl triflate or boron trifluoride dietherate (unpublished results).

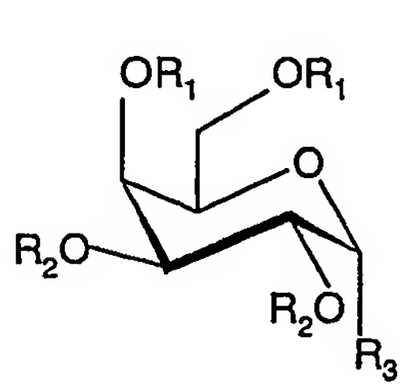
The problem underlying the present invention is to provide a cost effective method for the preparation of solasonine.

The present invention resides in the finding that the stereoselective β-glycosylation of solasodine may be achieved in high yields using specific galacto-pyranosyl donors. Preferably the reaction is carried out in the presence of a promoter.

Detailed description of the invention

It was unexpectedly found that by reacting a D-galacto-pyranosyl donor of the following formula 1

Formula 1



R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine

R_2 = Benzoyl, acetyl or pivolyl

R_3 = Halogen, SEt or SPh

Formula 1

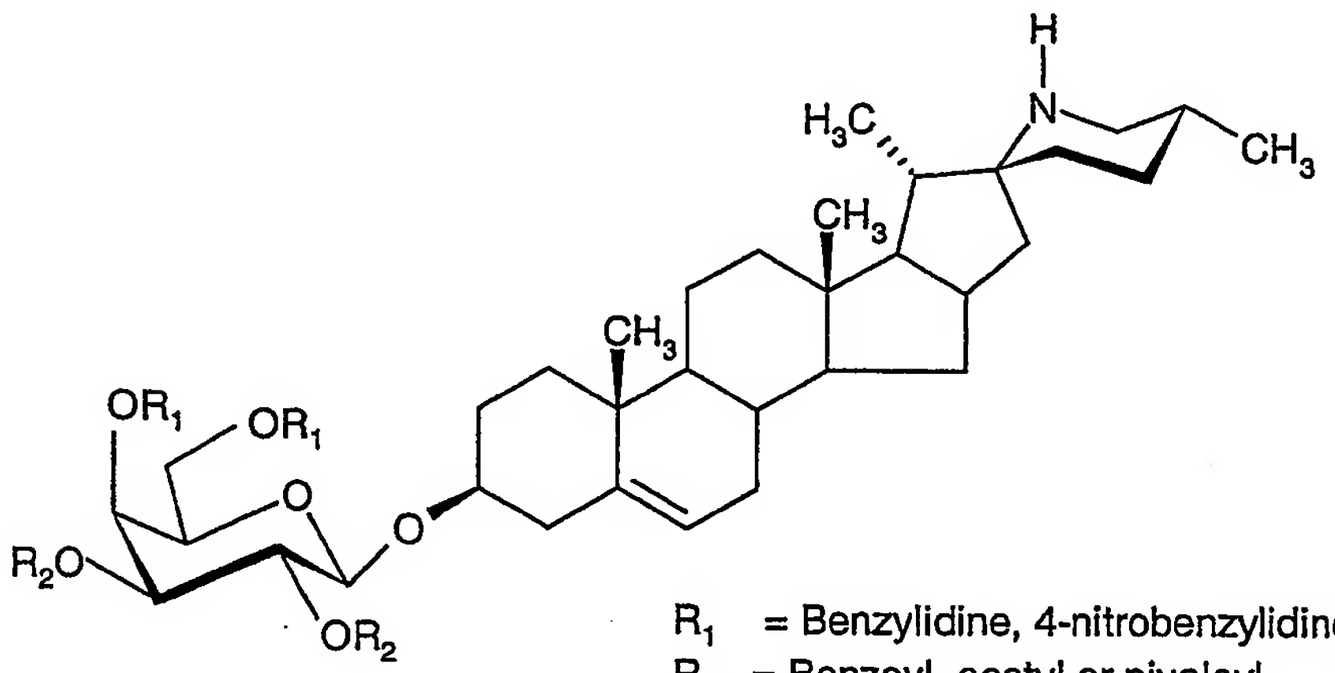
wherein each R_1 is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group

each R2 is the same or different and independently represents a benzoyl, acetyl or pivaloyl group and

R_3 is halogen, SPh or SEt

with solasodine the correspondingly protected β-glycoside of formula 2 could be obtained in high yield.

Formula 2



R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R_2 = Benzoyl, acetyl or pivaloyl

wherein each R_1 is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group and each R_2 is the same or different and independently represents a benzoyl, acetyl or pivaloyl group.

Preferably the reaction is carried out in the presence of a promoter.

Any conventional promoter as used in carbohydrate chemistry may be used.

The following promoters are particularly preferred:

Silver triflate, boron trifluoride diethyl etherate (-10°C), trimethylsilyl triflate bromide, N-iodosuccinimide, thiomethyl sulfonium triflate.

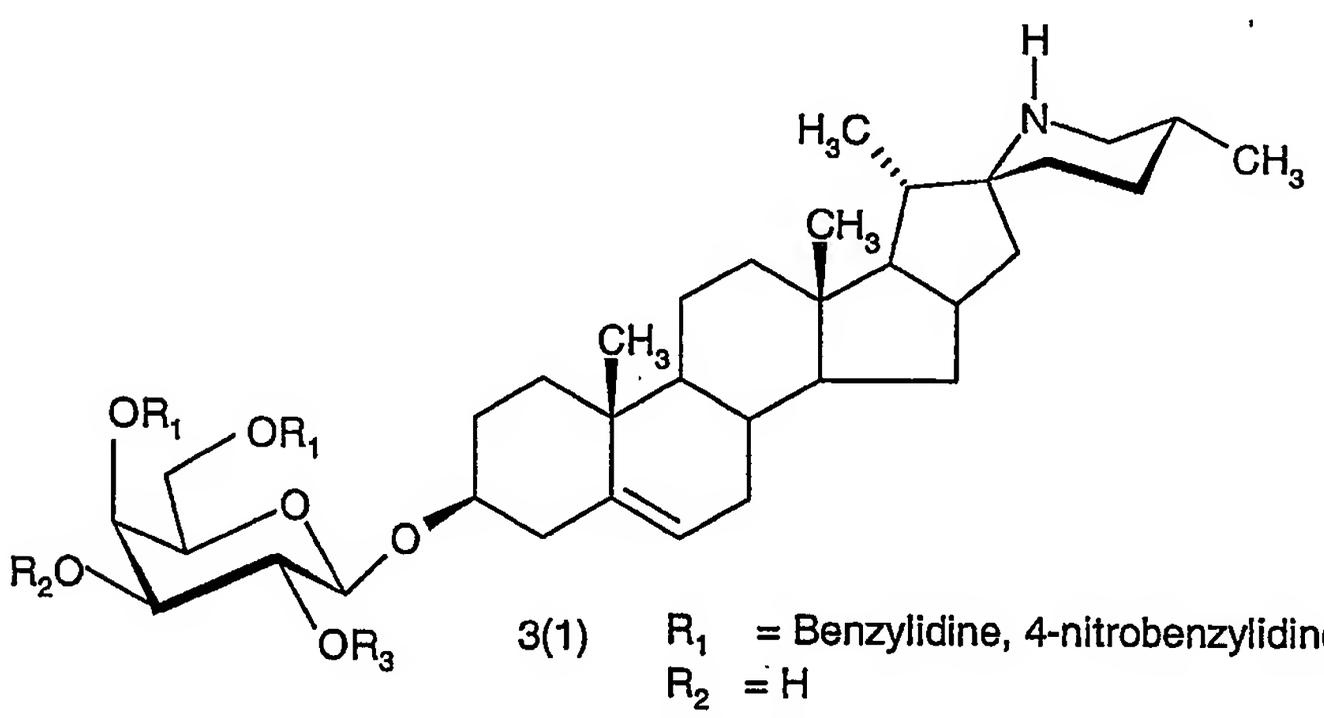
The reaction is preferably carried out using dichloromethane as the solvent. Preferably the reaction time is 30 min.-1 hr.

The desired end product solasonine may be prepared by partially deprotecting the β-glycoside of formula 2 to give intermediate formula 3(1) and then selectively

silylating one of the hydroxyl groups (OH-2 and OH-3) using tert-butyldimethylsilyl chloride, imidazole in DMF at 50°C.

However, due to the small selectivity between the OH-2 and OH-3 hydroxyl groups a mixture of OH-2 and OH-3 silylated protected β-glycosides are formed. The OH-3 silylated protected β-glycoside can mostly be precipitated from the mixture in methanol of the formula 3(2).

Formula 3



3(1) R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R_2 = H
R_3 = H

3(2) R1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R2 = Tert butyl silyl
R3 = H

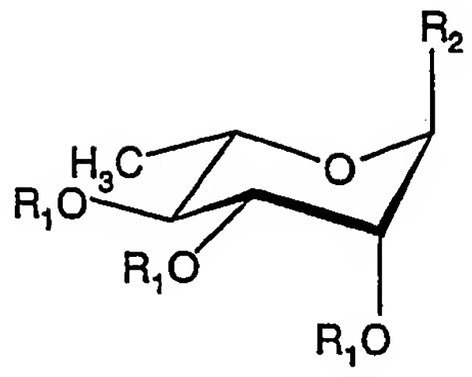
wherein each of R_1 is the same or different and represent independently from each other benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene,

R_2 is tert-butylsilyl or H and R_3 is H.

The OH-3 protected galactose-solasodine adduct is then glycosylated at the OH-2 with a suitable α-L-rhamnopyranosyl donor.

Suitable rhamnose donors include tri-O-benzolyl-α-rhamnopyranosly, tri-O-pivoloyl-L-rhamnopyranosyl, or tri-O-acetyl- 4- L-rhamnopyranosyl bromides of formula 4

Formula 4



R_1 = Acetyl, benzoyl or pivolyl

R_2 = Halogen, SEt or SPh

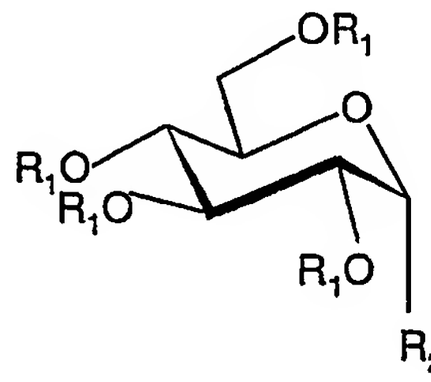
wherein each of R_1 is the same or different and independently represents acetyl, benzoyl or pivaloyl,

and R_2 is halogen or SEt, SPh

Deprotection of the tert-butylsilyl group at the OH-3 position using tetrabutylammonium flouride in THF and glycosidation with a suitable α-D-glucopyranosly donor,

wherein the D-gluco-pyranosyl donor is tetra-O-benzoyl-α-D-glucopyranosyl bromide, tetra-O-acetyl-α-D-glucopyranosyl bromide or tetra-O-pivoloyl-α-D-glucopyranosyl bromide, or a thio-glycoside of the general formula 5

Formula 5

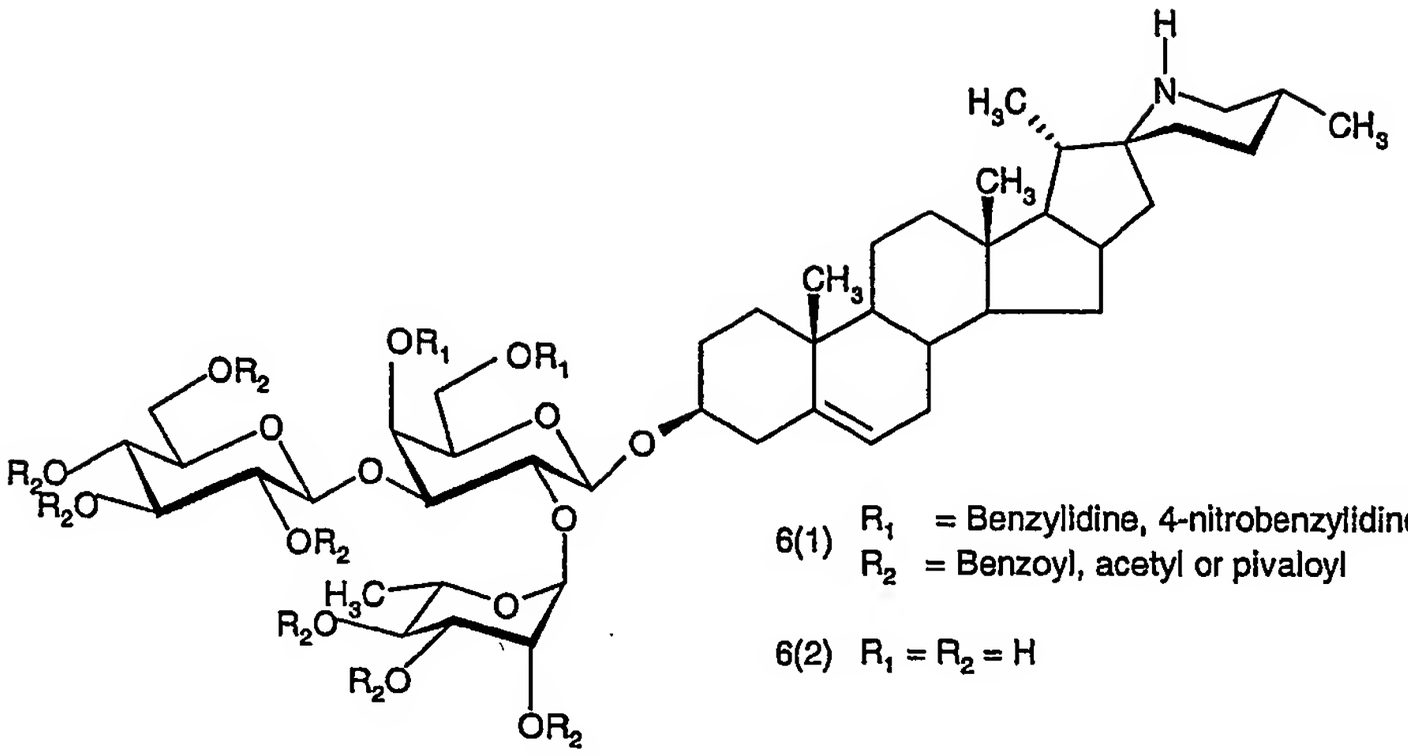


R_1 = Acetyl, benzoyl or pivolyl

R_2 = Halogen, SEt or SPh

wherein each of R_1 is the same or different an independently represents acetyl, benzoyl or pivaloyl and R_2 is halogen, SEt, SPh gives a fully protected solasonine of formula 6(1).

Formula 6



6(1) R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R_2 = Benzoyl, acetyl or pivaloyl

6(2) $R_1 = R_2 = H$

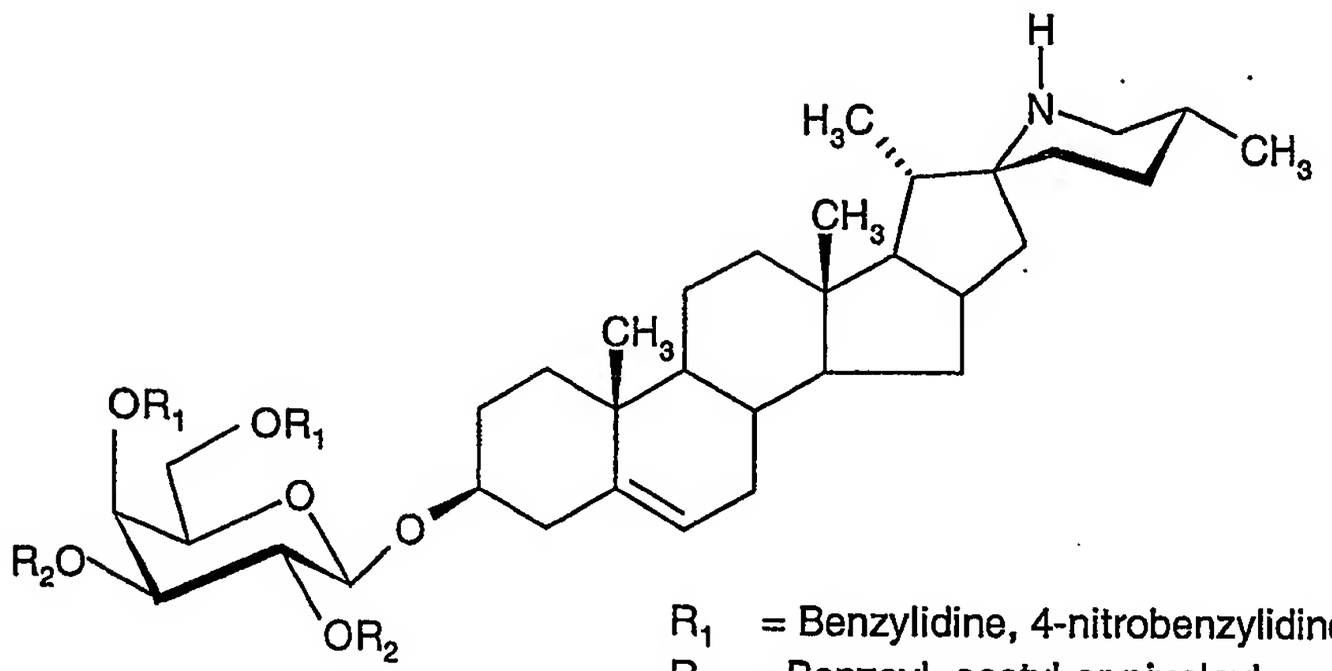
wherein each R_1 is the same or different and independently represents benzylidene, 4-nitrobenzyilidene or 4-methoxybenzylidene and each R_2 are the same or different and independently represent acetyl, benzoyl or pivaloyl.

The protected solasonine formula 6(1) may be de-acetalised using aqueous acetic acid at 70°C and de-esterified using sodium methoxide in methanol/dichloromethane mixture to give the fully deprotected solasonine formula 6(2) where in R_1 and R_2 are H.

Claims

1. A galactose-solasodine conjugate of the general formula 2 or a derivative thereof

Formula 2

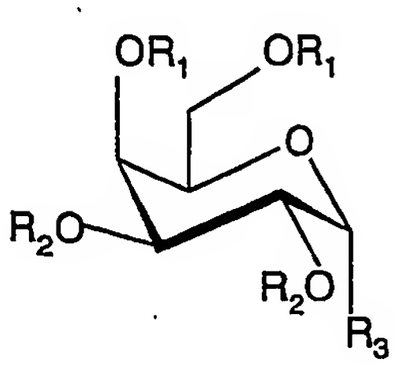


R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R_2 = Benzoyl, acetyl or pivaloyl

wherein each R_1 is the same or different and represents benzylidene, 4-nitro benzylidene or 4-methoxybenzylidene and each R_2 is the same or different and represents benzoyl, acetyl or pivaloyl.

2. A method for the preparation of the galactose-solasodine conjugate as defined in claim 1, comprising the reaction of solasodine with a galactopyranosyl donor of general formula 1

Formula 1



R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine

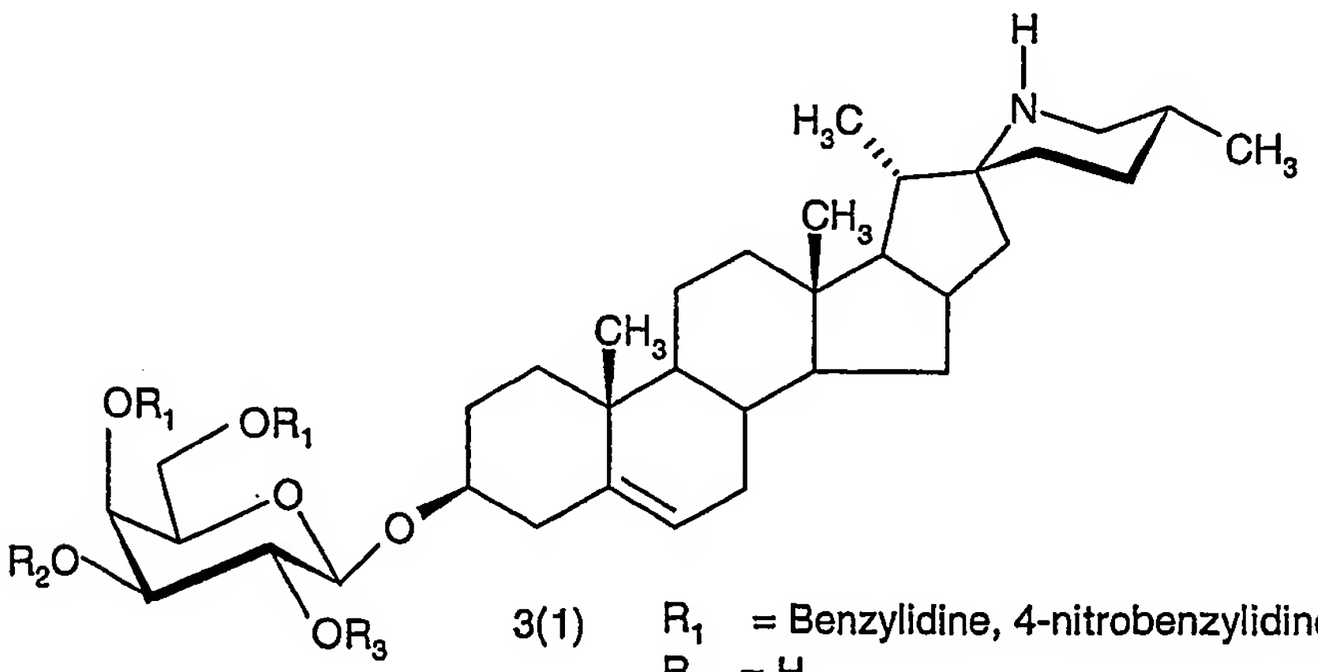
R_2 = Benzoyl, acetyl or pivolyl

R_3 = Halogen, SEt or SPh

wherein each R_2 independently represents a benzoyl, acetyl or pivaloyl group, and R_1 is benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene and R_3 is halogen, SE or SPh .

3. A method for the preparation of solasonine comprising the silylation of the diol of formula 3(1) to give a selectively silylated product in the OH-3 position 3(2)

Formula 3

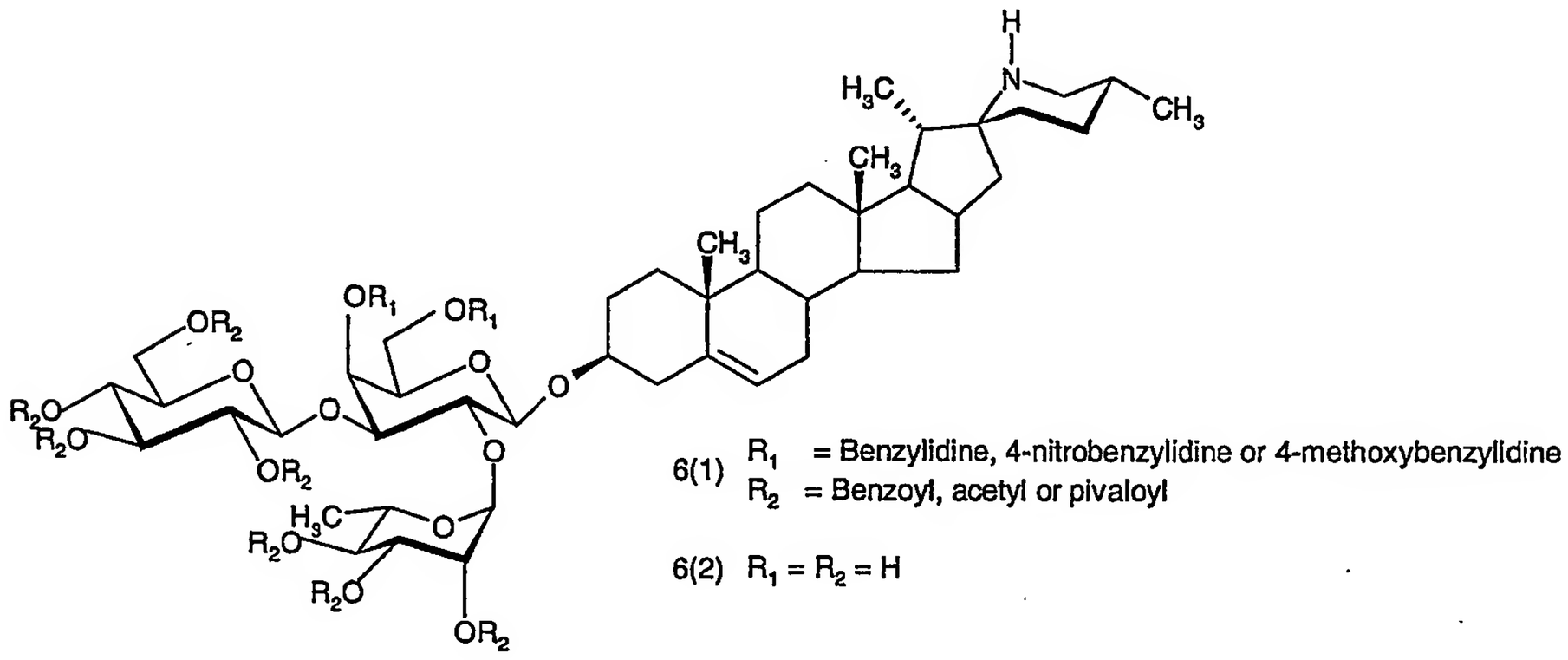


3(1) R_1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R_2 = H
R_3 = H

3(2) R1 = Benzylidine, 4-nitrobenzylidine or 4-methoxybenzylidine
R2 = Tert butyl silyl
R3 = H

wherein R_1 is represents either benzilidene, 4-nitrobenzilidene, or 4-methoxybenzylidene acetal protecting group by gylcosylation of OH-2 with an α-L-rhamnopyranosyl donor, followed by deprotection of the group on the hydroxyl (OH-3) and a further glycosylation of the hydroxyl (OH-3) with α-D-glucopyranosyl donor to yield the protected solasonine of formula 6(1) which is de-acetalised and de-esterified to yield a solasonine of formula 6(2).

Formula 6



4. The method according to claim 3, wherein the D-gluco-pyranosyl donor is tetra-O-benzoyl-α-D-glucopyranosyl bromide, tetra-O-acetyl-α-D-gluco-pyranosyl bromide or tetra-O-pivoloyl-α-D-glucopyranosyl bromide.

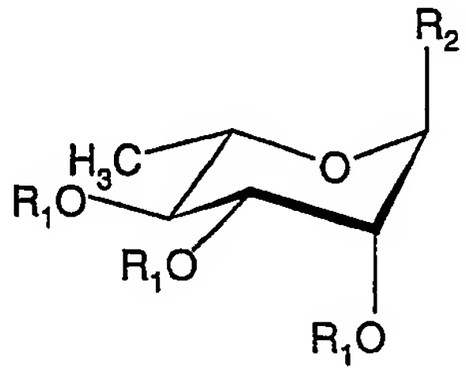
5. The method according to claim 2-4, wherein the glycosylation reaction is carried out in the presence of a promoter selected from silver triflate, boron trifluoride diethyl etherate, trimethylsilyl triflate bromide, N-iodosuccinimide or dimethyl thiomethyl sulfonium triflate.

6. The method of claim 3, wherein the protected glycoside is deprotected in methanol-dichloromethane solution by treatment with sodium methoxide, followed by neutralization with a mild acid ion-exchange resin.

7. The method of claim 3-6, wherein the hydroxyl groups (OH-4 and OH-6) are protected by acetalisation with Benzaldehyde dimethoxy acetal in DMF and a catalytic amount of para-toluene sulphonic acid.

8. The method of claim 3-7, wherein the rhamnose donor is tri-O-benzoyl-α-L-rhamnopyranosyl bromide, or a thio-glycoside of the general formula 4

Formula 4



R_1 = Acetyl, benzoyl or pivolyl

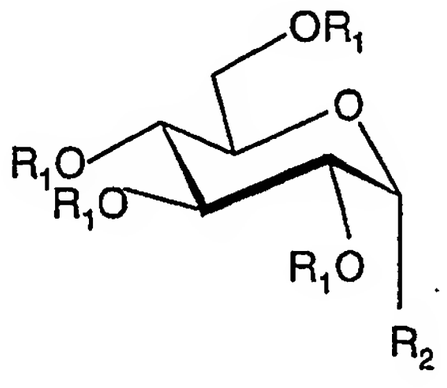
R_2 = Halogen, SEt or SPh

wherein each of the R_1 is the same or different and each independently represent benzoyl, acetyl or pivaloyl and

R_2 is halogen, SEt or SPh.

9. The method of claim 3-8, wherein the glucose donor is tetra-O-benzoyl-α–D-glucopyranosyl bromide, or a thio-glycoside of the general formula 5

Formula 5



R_1 = Acetyl, benzoyl or pivolyl

R_2 = Halogen, SEt or SPh

wherein each of R_1 is the same or different and independently represents benzoyl, acetyl or pivaloyl and R_2 is SEt, SPh or halogen

10. The method of claim 3-9, wherein the protected solasonine is de-acetalised and de-esterified by treatment with 80% acetic and then sodium methoxide solution in methanol-dichloromethane, followed by neutralization mild acid ion-exchange resin.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004629

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07J43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FEWELL A M ET AL: "INTERACTIONS BETWEEN THE GLYCOALKALOIDS SOLASONINE AND SOLAMARGINE IN RELATION TO INHIBITION OF FUNGAL GROWTH" PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 37, no. 4, 1994, pages 1007-1011, XP008011328 ISSN: 0031-9422 page 1010, left hand col., last paragraph	1-10
Y	WO 03/018604 A (GLYCOMED SCIENCES LTD ; SHAHID MOHAMMED (GB)) 6 March 2003 (2003-03-06) Step C, E, F	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2004	25/08/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Wörth, C

International Application No
PCT/EP2004/004629

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LI B ET AL: "An improved synthesis of the saponin, polyphyllin D" CARBOHYDRATE RESEARCH, ELSEVIER SCIENTIFIC PUBLISHING COMPANY. AMSTERDAM, NL, vol. 331, no. 1, 9 March 2001 (2001-03-09), pages 1-7, XP004317141 ISSN: 0008-6215 Scheme 1	1-10
Y	L. F. AWAD ET AL.: "A synthesis of 3-O-(alpha-D-mannopyranosyl)-D-mannose and its protein conjugate" CARBOHYDRATE RESEARCH, vol. 122, no. 1, 1983, pages 69-79, XP002292126 example 12	1-10
Y	A. FÜRSTNER ET AL.: "Ring-closing alkyne metathesis. Application to the total synthesis of sophorolipid lactone" J.ORG.CHEM., vol. 65, 2000, pages 8758-8762, XP002292185 example 16	1-10
Y	FIGUEROA-PEREZ S ET AL: "Synthesis of a sialyl-alpha-(2->6)-lactosamine trisaccharide with a 5-amino-3-oxapentyl spacer group at C-1<I>" CARBOHYDRATE RESEARCH, ELSEVIER SCIENTIFIC PUBLISHING COMPANY. AMSTERDAM, NL, vol. 317, no. 1-4, 30 April 1999 (1999-04-30), pages 29-38, XP004179992 ISSN: 0008-6215 examples 8-10	1-10
A	SUGIYAMA S DIAKUR J M: "A convenient preparation fo glycosyl chlorides from Aryl/Alkyl thiogl" ORGANIC LETTERS, ACS, WASHINGTON, DC, US, vol. 2, no. 17, 2000, pages 2713-2715, XP002956970 ISSN: 1523-7060 examples 25-28	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/004629

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03018604 A	06-03-2003	CA 2458183 A1	06-03-2003
		WO 03018604 A1	06-03-2003
		EP 1421100 A1	26-05-2004